

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231

on 5-29-01

(Date of Deposit)
Janice T. Staton
Name and Reg. No. of A
Janice T. Staton
Signature
5/29/01
Date of Signature

Express Mail #EL394336976US

ANTI-ALLERGIC PHARMACEUTICAL COMPOSITION CONTAINING AT LEAST ONE ALLERGEN AND AT LEAST ONE ANTIHISTAMINE COMPOUND

The present invention relates to new pharmaceutical compositions for the prevention and treatment of allergies. Allergies are a scourge which affects 25% of the world's population. This number is on the increase in connection with growing environmental toxicity (dust, food, motor vehicles). In addition, a person's risk of suffering from allergy is increased if there is a previous family history of allergy.

The biological mechanism of allergies may be described as an abnormally amplified reaction to the entry of an allergen into the body. The following events account for the reaction:

- identification of the allergen by the body,
- secretion of cytokines in response to allergen penetration,
- conversion of Th1 cells into Th2 cells, with the production of clones specific to the antigen,

- the Th2 cells synthesize interleukins 4 and 13, responsible for aggravation of the allergic symptoms through an upsurge in IgE synthesis

5 - the terminal phase of the reaction is the release of histamine and serotonin having a recruiting effect on the Th2 clones.

- toxic and inflammatory self-maintaining reaction, even without any antigen stimulation.

10 The antigen-presenting cells (APCs: macrophages, dendritic cells, B-lymphocytes) take part in the reaction of hypersensitivity through basic cell cooperation carrying the immune reaction further. Allergies belong to the nonself class of defence mechanisms. The main
15 allergens are acarids (dust mites) (80%) and pollens (20%).

20 The self-stimulating reactions of specific APC clones have an effect on the general rate of release of histamine and serotonin leading to an aggravation of the general clinical symptomatology.

25 The recruitment level of new IgE-secreting cells is thereby increased facilitating the explosion of clinical signs when a new allergen penetrates inside the body. This can be seen in atopic persons in whom allergic reactions are severe owing to the high level of Th2 clones promoting the synthesis of IgE.

The general reaction observed subsequent to the penetration of the new allergen is not due to its toxicity but simply to the fact that the triggering level of allergic phenomena is very low, helped by other 5 sensitisations.

An allergy is a reaction due to hypersynthesis of IgE immunoglobulins. The inflammatory reaction chiefly affects the respiratory and ENT spheres, with pathological 10 focalisation at the nose, lungs and skin. Pathologies associated with the allergy are invalidating and suffer from the lack of efficacy of conventional treatment. There is no preventive strategy and curative means are insufficient or ill used.

15 The usual treatment of allergic disease consists, during a first phase, of identifying the allergen responsible: dust mites, pollen, mould, food. The second phase comprises removal measures. The third phase 20 treatment phase focuses on the target organ which appears to be symptomatic: ENT treatment for rhinitis, anti-asthmatic treatment if the affected sphere is respiratory, dermatological treatment if the affected areas are skin areas.

25 In the event of failure of the preceding measures, individual or complementary treatment may be offered through the choice of a specific immunotherapy (specific pollen, specific acarid, specific mould). The complexity

of the treatment instituted makes it difficult to follow.
A succession of treatments is a patent factor of failure.

The purpose of the present invention is precisely to
5 offer new means of treating allergies that are both
preventive and curative.

This purpose is achieved by treating the two main
sides of the immune reaction:

10 - firstly the upstream part of the immune response
which, after presenting the antigen to the APCs leads to
increased synthesis of the IgEs responsible for the self-
recruiting of the immunity cells, and
- secondly, the downstream side of the immune
15 response which leads to release of the preformed
mediators, essentially histamine, responsible for the
final clinical outcome.

The optional combined use of an inhibitor of
histamine synthesis makes it possible to reduce the
20 concentration of the latter and therefore to improve the
therapeutic efficacy of the pharmaceutical composition
according to the invention.

The present invention concerns a anti-allergic
pharmaceutical composition containing at least two active
25 agents chosen among : (i) one allergen, (ii) one
antihistamine compound, (iii) one inhibitor of histamine
synthesis, said active agents being associated in said
composition with a pharmaceutically acceptable vehicle.

Consequently, the subject of the invention is more particularly an anti-allergic pharmaceutical composition containing (i) at least one allergen and (ii) at least one antihistamine compound, and optionally (iii) at least one inhibitor of histamine synthesis, in a pharmaceutically acceptable vehicle.

A first preferred form of anti-allergic pharmaceutical composition according to the invention contains (i) at least one allergen and (ii) at least one antihistamine compound, in a pharmaceutically acceptable vehicle, enabling release of the peptides and other chemical substances in independent manner at galenic level.

Advantageously, said allergen is chosen from among the major antigens or mixture of major antigens of acarids able to induce an immune reaction. Indeed, the research conducted within the scope of the invention consisted of using ubiquitous antigens of acarids. These antigens are present in substantial quantity in the environment and are the cause of the development of allergic reactions in the world. Two acarids, *D. Pteronyssinus* (DP) and *D. Farinae* (DF) are the most represented in the world environment.

The invention most particularly gives consideration to a cystine protease as allergen, the carrier of antigenicity which is 90 % identical for these two acarids. The epigenic and amino acid sequences of the cystine protease of *D. Pteronyssinus* (DP) are shown in the

list of appended sequences given respectively under numbers SEQ ID NO : 1 and SEQ ID NO : 2.

The allergens used in the compositions of the invention may either be extracts obtained from crude biological material, or wholly or partly purified proteins 5 optionally produced by genetic engineering or by peptide synthesis.

Therefore the invention further concerns as allergen the peptide epitopes of cystine protease. Three epitopic parts have been identified which form triggering agents 10 for the immune response. These are the three peptides with the following sequences:

RMQGGCGSCN (SEQ ID NO : 3)

QPNYHAVNIV (SEQ ID NO : 4)

15 WTVRNSWDT (SEQ ID NO : 5)

and their possible analogues.

The sequences of the protein epitopes cited above may contain primers and supplementary amino acid sequences or substitutions facilitating their adhesion to the Major 20 Histocompatibility Complex (MHC).

The invention gives special consideration to pharmaceutical compositions containing at least one of these peptides as allergen.

25 These peptide epitopes are strictly identical in DF and DP, and in other acarids since they are carriers of the enzyme function of cystine protease. Their lipophilicity and the fact that they tolerate the enzyme function, account for the fact that these epitope parts are constant

from one species of acarid to another and that they are the site of a general immune response.

The use of these parts, either in the form of cycled proteins, or in epigenic form, even in their RNA form,
5 must induce tolerance to the natural antigen and reduce the general level of the immune response upstream.

Cyclising the epitopes and/or inclusion of the epigenic patterns in a longer sequence makes it possible to improve the presenting of the antigens to the T-
10 lymphocytes. This improved presentation will allow presentation of the antigens and epitopes to the MHC and thereby trigger the immune tolerance response. The antigens must previously be rearranged by the APCs. The simple epitopic form does not allow rearrangement by the
15 APCs since, as a general rule, only a protein longer than 10 amino acids may be cut and presented by the APCs to the T-lymphocytes.

These peptides may be associated with any
20 pharmaceutically acceptable vector, of phospholipid type for example.

If epigens are involved, the latter may be primed by the following nucleotide sequence: 5'GC GG CG CG 3' (SEQ ID
25 NO : 6).

The controlled reaction of the TH2/TH1 switch induced by this protein or its epigen may also be achieved using other methods, in particular with the nucleotide primers according to the following sequence 5'TG AG CG GG CG GG CG 3' (SEQ

ID NO : 7), and using any other method allowing upstream control of the TH2/TH1 switch.

It is therefore possible to integrate the epigens corresponding to the epitopes of DP/DF with a nucleotide primer sequence of sequence (SEQ ID NO : 7) by alternating said sequence (SEQ ID NO : 7) and an epitope such as to integrate the three major epitopes of DP/DF either together or separately.

The integration of the epitopes together leads to obtaining a group made up of a nucleotide primer sequence (SEQ ID NO : 7) a first major epitope, a nucleotide primer sequence (SEQ ID NO : 7), a second major epitope, a nucleotide primer sequence (SEQ ID NO : 7), a third major epitope.

The integration of epitopes separately leads to mixing three groups each made up of a nucleotide primer sequence (SEQ ID NO : 7) and a major epitope. This integration of the epitopes with a nucleotide primer sequence according to the following sequence (SEQ ID NO : 7) must improve the efficacy with which the DP/DF epigens are presented to the T-lymphocytes. With this improved presentation, the DP/DF epigens will stimulate the TH1 switch and therefore reduce the level of the allergic response.

The use firstly of these epitopes, or of a solution enabling the TH1/TH2 switch such as the nucleotide primers of sequence (SEQ ID NO : 7), and secondly their association with an antihistamine compound and optionally with an inhibitor of histamine synthesis provide an

efficient, innovative solution for the prevention and treatment of allergies.

Consequently, the compositions of the invention comprise an efficient quantity of at least one allergen such as defined above without predicting the role of this allergen in the patient's symptomatology.

With this approach it is possible to have global access to the allergic illness without giving consideration to the specificity of the allergen. Indeed with the composition of the invention it is possible to treat a level of immune reactivity and not to propose a specific immunotherapy.

The use of the allergen, under the different forms described above, in the compositions of the invention means that it is possible to induce tolerance to the natural antigen and to reduce the general level of immune response upstream. However, as mentioned previously, the allergen cannot alone cure the allergy since the toxic, inflammatory terminal reaction subsists which is self-maintaining without antigen stimulation. This reaction must also be treated by blocking the terminal phase of the allergy. Blocking the histamine receptors is the main effector mechanism. This blocking must be made over a time interval that is sufficiently long for there to be a negative feedback on the synthesis of these receptors. Antihistamines are anti-receptor molecules of choice to block this terminal reaction. Therefore, the compositions of the invention, in addition to the allergen, contain an

antihistamine compound and optionally an inhibitor of histamine synthesis.

As antihistamine compounds, mention may be made of: brompheniramine, cetirizine, fexofenadine, cyproheptadine, dexchlorpheniramine, hydroxizine, ketotifene, loratadine, mequitazine, oxotomide, mizolastine, ebastine, astemizole, carbinoxamide, alimemazine, buclizine, cyclizine hydrochlorate, doxylamine.

10 As indicated above, the allergy is also accompanied by increased synthesis of histamine, which also causes self-maintaining of the terminal inflammatory reaction. This histamine synthesis may possibly be controlled, in order to improve the efficacy of the previously proposed
15 pharmaceutical composition. This control has recourse to the inhibition of histamine synthesis. Consequently, the compositions of the invention contain an efficient quantity of an antihistamine compound which may optionally be associated with an inhibitor of histamine synthesis.
20 Therefore, blocking of the terminal histamine effector mechanisms will provide efficient control over the final cascade of the allergic reaction. The terminal route for the synthesis and stimulation of histamine receptors must therefore be blocked in global manner for the composition
25 to have improved efficacy.

A particular form of implementation of the invention consists in a anti-allergic pharmaceutical composition containing at least one antihistamine compound and at

least one inhibitor of histamine synthesis, said compounds being associated in said composition with a pharmaceutically acceptable vehicle.

As inhibitors of histamine synthesis, mention may be
5 made of an inhibitor of histidine decarboxylase as such tritoqualine.

By preventing histamine synthesis, the inhibitor of histidine decarboxylase increases the efficiency of the
10 composition in its action on the downstream side of the allergies biological mechanism by complementing the antihistamine compound.

The compositions of the invention provide a new allergen approach providing preventive vaccination against
15 the development of allergic illnesses. The objective being to restore a silent defence homeostasis to the body in relation to its environment.

The compositions of the invention contain a quantity of allergens in the order of 1 to 1500 µg and
20 advantageously from 10 to 150 µg. Concerning the peptides, each one is advantageously present in proportions in the region of 1 to 1500 µg so as to slow down the immunological response leading to increased IgE synthesis.

25 The antihistamine compound is present in the compositions of the invention in a proportion of the order of 1 to 2000 mg.

In the case of a composition according to the invention containing a antihistamine compound and an inhibitor of histamine synthesis, these compounds are present in a proportion of the order of:

5 - 5 to 200 mg of antihistamine compound,
 - 10 to 300 mg of an inhibitor of histidine
decarboxylase as such tritoqualine.

The compositions of the invention may be presented in
10 a form for transdermal application, for example an
ointment for children, a form for oral administration, for
example a slow release product, or in gastro-resistant
tablet form or gum form. They may also be in spray or eye
lotion form, or galenic forms with programmed mucosal and
15 secondarily *per os* disintegration.

Therefore the different compositions of the invention
can be administered by several routes chosen in accordance
with the patient's pathological profile and age. For
20 children, the patch form, syrup form or tablets to be
dissolved in the mouth. The other forms, eye lotion or
injection may also be used. In adults all galenic forms
can be contemplated.

The advantage of a coupled form also provides
25 simplicity of treatment, patient compliance with the
simplified treatment and therefore a more successful
outcome.

This solution also makes it possible to prevent the
allergic illness and not only patent pathological

conditions. Children of allergic parents could be the major target of this preventive treatment. The result would be shorter hospital stays, fewer antibiotic treatments, and improved quality of life. Indeed the
5 TH2/TH1 switch must occur as early as possible in order to be effective, since in infants it is the TH2 route which predominates, responsible for hyper-response to the environment. The TH2/TH1 switch must occur early for its duration to be as long as possible, since antigenic
10 stimulation by the antigens of the environment (dust mites and bacteria) are stimulators of the TH2 route.

Therefore the pharmaceutical composition of the invention is particularly useful for the preparation of a
15 medicinal product intended to treat allergic hypersensitive reactions.

Advantageously, the pharmaceutical composition of the invention is in a galenic form with programmed mucosal or sublingual and secondarily *per os* disintegration.
20

The pharmaceutical composition of the invention is also useful for the preparation of a medicinal product intended to treat or prevent allergic hypersensitivity reactions, to treat or prevent allergic asthma, allergic
25 rhinitis and atopic and allergic eczema.

Finally the pharmaceutical composition of the invention is particularly useful for the preparation of a

medicinal product intended to treat or prevent allergic symptoms in children, infants and adults.

Other advantages and characteristics of the invention
5 will become apparent on reading the clinical observations made in the treatment of allergic patients as recorded in the table given below.

These observations were made on approximately one
10 hundred patients who were given a composition of the invention associating at least one allergen and an antihistamine compound.

Patient age ranged from 7 to 60 years. They all presented with at least one positive dust mite or pollen
15 prick test, and symptomatology of rhinitis or asthma of at least one year's onset.

The pathological profile of the patients was classified according to the following typology comprising three descriptive categories: inflammation, secretion and
20 the figured element.

- Only clinical examination was used to classify inflammation. It was considered that there was inflammation if examination of the mucosa or target organs showed redness confirming an inflammatory phenomenon,

25 - Secretion concerned the observation of an exudate whether purulent or non-purulent affecting a target organ (mucosa, skin, etc...).

- The figured element concerned a change in the structure of the organ under consideration, which may

occur in several pathological forms. Consideration was only given to the existence of a change without going into the detail of this change.

The grading of pathological severity used a scale of 5 1 to 4 measuring intensity as a fraction 1/4 or 1/2 or a whole number.

Therefore, according to this grading, an assessment of 1/4 denotes target organ impairment of between 0 and less than 1/4. An assessment of 1/2 denotes target organ 10 impairment of between 1/4 and one half; an assessment of 3/4 denotes target organ impairment of more than one half and less than 3/4; an assessment of 1 denotes impairment of more than 3/4.

A first category of target organs was graded 15 according to this typology. It comprises the eyes, nose, pharynx, larynx and the skin.

In respect of the lungs, rating used the results of functional respiratory investigation expressed as a percentage relative to the normal value (using an 20 international classification method taking into account age and size in particular).

The patients were given follow-up with at least one consultation at 2 months, 8 months, 12 months, 24 months. The course of the treatments followed and the number of 25 units taken were analysed.

Table 1 below gives a clear indication of the very positive results obtained after a treatment time of approximately 8 months. A distinct improvement was noted

in the pathological condition of the patients, with a drop in the overall clinical score for severity falling from an average value of 9.56 to 2.47, the standard deviation decreasing from 1.15 to 0.53, confirming the efficacy of
 5 the treatment in all patient age and sex groups. The mean number of affected target organs fell from 3.69 to 1.73, while the standard deviation in the number of target organs affected was reduced from 0.49 to 0.41.

10 Table I

Patient reference	Sex	Date of birth	Date of initial consultation	N° of tests +	Initial consultation		3 rd consultation after 8 months' treatment	
					N° of target organs affected	Total clinical score	N° of target organs affected	Total clinical score
1	M	1964	1996	3	3	7	2	2
2	F	1936	2000	4	3	6	1	2
3	F	1944	1993	8	4	10	2	2
4	F	1974	1997	8	4	9	1	3
5	F	1950	1997	8	4	9	2	3
6	M	1960	1997	7	4	8	1	2
7	F	1944	1996	4	3	6	2	2
8	F	1963	1993	4	5	10	1	2
9	M	1988	1993	7	4	8	2	2
10	M	1991	1993	3	4	9	1	2
11	M	1971	2000	6	3	9	1	2
12	M	1948	2000	3	4	9	1	2
13	M	1929	2000	3	3	7	2	2

					Initial consultation		3 rd consultation after 8 months' treatment	
					Patient reference	Sex	Date of birth	Date of initial consultation
14	M	1953	1999	5	4	9	1	1
15	F	1932	1994	10	4	10	1	2
16	F	1934	1996	8	6	11	2	2
17	F	1982	1993	5	4	10	2	2
18	F	1968	1994	4	4	10	2	2
19	M	1996	1996	4	4	10	1	3
20	F	1991	1997	5	4	10	2	3
21	F	1990	1996	7	3	8	1	2
22	F	1949	2000	4	4	8	2	3
23	M	1995	2000	3	2	6	1	2
24	F	1961	1994	8	3	8	1	2
25	M	1987	1994	7	4	9	2	3
26	F	1991	1995	8	3	8	1	2
27	M	1967	1994	7	3	9	2	2
28	M	1989	1994	7	4	9	2	3
29	M	1947	1999	5	4	9	2	2
30	F	1920	1999	2	3	8	1	2
31	F	1963	1997	6	4	9	2	2
32	M	1979	1998	4	4	9	1	2
33	F	1983	2000	3	3	8	2	2
34	M	1996	1999	7	4	8	2	2
35	F	1946	1995	7	3	8	2	3
36	F	1958	1995	5	4	10	2	2
37	F	1946	1997	6	4	11	2	2
38	F	1965	1993	3	3	9	1	2
39	M	1973	2000	7	4	9	2	2
40	M	1957	1995	5	4	9	2	2
41	F	1942	1995	8	4	9	2	2

Patient reference	Sex	Date of birth	Date of initial consultation	N° of tests +	Initial consultation		3 rd consultation after 8 months' treatment	
					N° of target organs affected	Total clinic al score	N° of target organs affecte d	Total clinic al score
42	F	1933	1999	4	3	9	1	3
43	F	1959	1999	4	3	8	2	3
44	F	1965	1999	3	4	10	2	2
45	F	1944	1999	3	4	10	2	3
46	F	1942	1996	6	4	11	1	3
47	F	1948	1997	6	4	11	2	3
48	F	1963	1999	4	4	10	2	2
49	M	1981	1999	5	4	12	2	2
50	M	1995	2000	5	4	12	2	2
51	M	1989	1999	5	4	10	2	2
52	M	1997	1998	4	4	10	2	3
53	F	1997	1998	5	4	9	1	3
54	F	1995	1997	4	4	10	2	3
55	F	1984	1993	3	3	9	1	2
56	M	1969	1996	10	4	12	2	3
57	M	1951	1996	11	4	11	2	2
58	M	1992	1997	5	4	11	2	3
59	M	1975	1994	4	3	9	1	2
60	M	1977	2000	5	4	12	2	3
61	M	1989	1993	5	4	12	2	3
62	M	1994	1998	8	4	11	2	3
63	F	1993	1998	7	4	10	2	2
64	F	1988	1993	3	3	9	2	3
65	F	1940	1999	4	4	11	2	2
72	F	1951	2000	6	4	11	2	3
73	F	1956	1999	5	4	11	2	3
74	M	1982	1994	4	3	9	2	3
75	F	1944	1998	3	4	12	2	2

Patient reference	Sex	Date of birth	Date of initial consultation	N° of tests +	Initial consultation		3 rd consultation after 8 months' treatment	
					N° of target organs affected	Total clinic al score	N° of target organs affected	Total clinic al score
76	F	1992	1997	7	3	9	2	3
77	M	1997	1993	4	3	9	1	3
78	F	1955	1997	5	4	10	2	3
79	F	1996	1999	4	3	8	2	3
80	F	1936	1993	5	4	10	1	2
81	M	1949	1998	5	3	10	2	2
82	M	1966	1993	4	3	9	2	2
83	F	1963	2000	5	4	10	1	2
84	F	1954	1993	5	4	11	2	2
85	F	1995	2000	4	3	9	2	3
86	M	1988	1994	6	3	8	2	2
87	F	1969	1997	6	4	9	2	3
88	M	1963	1993	5	4	9	2	2
89	M	1994	1998	7	4	10	1	3
90	F	1992	1997	6	3	9	3	3
91	M	1988	1999	6	4	11	2	3
92	M	1955	1993	6	4	11	2	3
93	M	1944	1996	7	4	13	2	3
94	M	1986	1994	6	4	12	2	3
95	M	1954	1996	6	4	11	2	3
96	F	1989	1993	6	4	12	2	2
97	M	1965	1995	6	3	8	2	3
98	M	1986	1994	4	3	9	2	4
99	F	1956	1995	4	4	10	2	3
100	F	1944	1993	2	3	9	1	3
101	F	1995	1998	5	3	9	2	4
102	M	1960	1996	3	3	8	2	3
103	F	1928	1995	6	4	10	2	3

Table II below gives the mean clinical score and the standard deviation in the scores obtained.

5 Table II

			INITIAL VISIT	VISIT AT 8 MONTHS
MEAN CLINICAL SCORE			9.56	2.47
STANDARD DEVIATION IN SCORES			1.15	0.53

Table III below illustrates the average number of target organs affected and the standard deviation in the number of target organs affected.

10

Table III

			INITIAL VISIT	VISIT AT 8 MONTHS
MEAN N° OF AFFECTED TARGET ORGANS (T.O.)			3.69	1.73
STANDARD DEVIATION IN N° AFFECTED T.Os.			0.49	0.41